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RNA cleavage and chain elongation by *Escherichia coli* DNA-dependent RNA polymerase in a binary enzyme·RNA complex

(gene regulation/cleavage/RNA binding)

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ABSTRACT **In the absence of DNA, *Escherichia coli* RNA polymerase (EC 2.7.7.6) can bind RNA to form an equimolar binary complex with the concomitant release of the σ factor. We show now that *E. coli* RNA polymerase binds at a region near the 3′ terminus of the RNA and that an RNA in such RNA·RNA polymerase complexes undergoes reactions previously thought to be unique to nascent RNA in ternary complexes with DNA. These include GreA/GreB-dependent cleavage of the RNA and elongation by 3′-terminal addition of NMP from NTP. Both of these reactions are inhibited by rifampicin. Hence, by several criteria, the RNA in binary complexes is bound to the polymerase in a manner quite similar to that in ternary complexes. These findings can be explained by a model for the RNA polymerase ternary complex in which the RNA is bound at the 3′ terminus through two protein binding sites located up to 10 nt apart. In this model, the stability of RNA binding to the polymerase in the ternary complex is due primarily to its interaction with the protein.**

Transcription is an important target for the control of gene expression in eukaryotes and prokaryotes. Regulation occurs at all phases of the transcription process: promoter binding and RNA chain initiation, elongation, and termination. One regulatory target, the elongation or ternary complex, consists of template DNA, RNA polymerase (EC 2.7.7.6), and the newly transcribed RNA. Two different models for the structure of the elongation complex have been proposed. One model, proposed by Gamper and Hearst and refined by von Hippel and coworkers (1–4), views binding of the RNA to the complex as due to DNA–RNA interactions within a 12-bp DNA·RNA hybrid formed at the 3′ end of the nascent RNA. In the second model, it is proposed that RNA–protein interactions play an important role in both the mechanism and the regulation of the enzyme and that these interactions are mediated by a specific RNA binding site(s) on the enzyme (5–11). In this model, any hybrid that may be present does not play a central role in binding of the RNA to the complex.

The fact that free RNA can bind to RNA polymerase is not novel (12, 13). Early studies demonstrated the specific binding of RNA to the enzyme by its inhibitory effect on transcription, as well as by numerous physical methods (14–16). That RNA interacts with a binding site distinct from the DNA binding site can be seen by the differential sensitivity of binding to the polyamine spermidine (15), the release of the σ subunit with RNA binding (17–19), and by the differential sensitivity of core subunits to protease cleavage (20–22). In the first modern quantitative studies on the interaction of tRNA and *Escherichia coli* RNA polymerase, Buc and coworkers (19, 23) showed, using a number of complementary assays, that different tRNAs each formed a 1:1 complex with core RNA polymerase in its monomer and dimer forms. In addition, they demonstrated that, although DNA binding decreases the binding of tRNA and vice versa, the binding sites for the two nucleic acids are distinct.

Recently, experiments designed to examine the physical and structural properties of ternary complexes have demonstrated a transcript shortening reaction *in vitro* (24) in which the 3′ end of the nascent RNA is endonucleolytically removed and released. The 5′-terminal fragment of the RNA remains bound to the ternary complex in an active form and can serve as a primer for continued elongation. This cleavage activity has been shown to be associated with at least three factors: GreA and GreB for *E. coli* RNA polymerase (25, 26) and TFIIS for eukaryotic RNA polymerase II (27–34). Cleavage has also been observed in ternary complexes formed with the DNA-dependent RNA polymerase from vaccinia, which contains a subunit with sequence homology to the eukaryotic factor TFIIS (35, 36).

Up to 17 nt of RNA can be excised as a unit from the 3′ end in this reaction, leaving the 5′ end stably bound and active (24–26, 29–31, 34, 36–40). Cleavage and release of such a long piece of the 3′-terminal portion of the nascent transcript poses a serious problem for the classical model of the ternary complex in which the stability of RNA binding is attributed to formation of a 12-bp DNA·RNA hybrid at the 3′ terminus. It seems more likely that a region of the RNA, well upstream of the immediate 3′ end of the RNA, must be bound to the enzyme by a specific RNA binding site or sites. If so, the factor-dependent cleavage reaction as well as the nucleotide addition reaction might well be expected to occur with binary complexes, containing only RNA and RNA polymerase, as well. We show here that this is the case.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids. *E. coli* strain AD8571 ΔGreA ΔGreB (*greA*$^-$, *greB*$^-$, *rpoB*$^+$) was a generous gift of Janet Newlands and Asis Das (University of Connecticut). Overexpressing plasmids pDNL278 and pGF296 containing *E. coli greA* and *greB* genes, respectively, were kindly provided by Robert Landick (Washington University, St. Louis). The plasmid pCPG1000 has been described by Reynolds and Chamberlin (11). pN25 was obtained from Hermann Bujard (European Molecular Biology Laboratory) and pM19 (41) was obtained from H. Heumann (Max Planck Institute, Munich). The PCR primers used will be described elsewhere (L. Hsu and M.J.C., unpublished work).

Buffers and Materials. BA85 nitrocellulose filters were obtained from Schleicher & Schuell. Radionucleotides were purchased from NEN. Nonradioactive nucleotides were purchased from Pharmacia LKB Biotech.

Standard transcription buffer contains 40 mM Tris–acetate at pH 8.0, 50 mM ammonium acetate, 20 mM potassium acetate, 4 mM magnesium acetate, and 5 mM 2-mercapto-

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ethanol. RNA elution buffer is 10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.5 M NaCl/0.1% SDS. *E. coli* RNA polymerase diluent (RNAP diluent) and polymerase storage buffer are described by Chamberlin *et al.* (42).

Protein Purification. *E. coli* DNA-dependent RNA polymerase was purified by the method of Burgess and Jendrisak (43). The holoenzyme was separated from core polymerase by chromatography either on phosphocellulose according to Gonzalez *et al.* (44) or on Mono Q (Pharmacia) according to Hager *et al.* (45). Activity was determined by the method of Chamberlin *et al.* (46). The percentage of active molecules was ≈60% and the purity was >95% as determined by SDS/PAGE and Coomassie R250 staining.

GreA protein was purified from JM109 bearing the overexpressing plasmid pDNL278, which contains the *greA* gene downstream of the inducible P_{tac} promoter; the purification will be published elsewhere (D.E.S.-C. and M.J.C., unpublished work). The purified protein was dialyzed against storage buffer [50 mM Tris·HCl, pH 8.0/1 mM EDTA/50% (vol/vol) glycerol/0.2 mM dithiothreitol] and stored at −20°C.

GreB was purified from JM109 bearing pGF296, which contains the *greB* gene downstream of the isopropyl β-D-thiogalactoside (IPTG) inducible promoter P_{tac}, using a modification of the methods used for the GreA purification. Purified protein was stored in storage buffer at −20°C. Dilutions of both GreA and GreB were done in storage buffer containing acetylated bovine serum albumin at 0.4 mg/ml.

Preparation of RNA. RNA was prepared in an extensive synthesis reaction (50–250 μl) as described by Arndt and Chamberlin (9), using a pCPG1000 *Sst* I digest or PCR-amplified templates of pM19 or pN25 to generate CPG79 RNA, M19-68 RNA or N25-50 RNA, respectively. Reaction products either were labeled at the 5′ end by including [γ-^{32}P]ATP (≈20,000 cpm/pmol) in the reaction mixture or were prepared without label. RNA was precipitated with ethanol and resuspended in 1/10 vol of formamide loading buffer and isolated on 7 M urea/15% polyacrylamide gels (19:1 acrylamide/bisacrylamide) in 1× GB (90 mM Tris–borate, pH 8.0/25 mM EDTA). RNA was visualized directly by autoradiography or indirectly by using labeled markers, excised, and eluted overnight in 300 μl of RNA elution buffer. Samples were precipitated with ethanol and resuspended in 10 mM Tris–acetate, pH 8.0/0.1 mM EDTA and stored at 4°C.

Mobility Shift Assays. Mobility shift assays were performed according to the method of Fried and Crothers (47). Briefly, CPG79 RNA (2–20 pmol, as indicated) was incubated with RNA polymerase (1–2 pmol, as indicated) in standard transcription buffer containing 0.1× RNAP diluent and 5% glycerol in a total volume of 25 μl. The reaction mixtures were incubated for 5 min on ice and 15-μl samples were loaded directly on running 4% polyacrylamide gels (250 V) at 4°C. The electrophoresis buffer was 1× GB and gels were pre-run for at least 1 hr prior to the application of sample. The relative amount of bound and free RNA was determined by analysis on a Molecular Dynamics PhosphorImager.

RNA Cleavage Assay. Standard RNA cleavage assays were performed by incubating end-labeled RNA (0.2 μM) with GreA$^-$ GreB$^-$ RNA polymerase (1.0 μM) and either GreA or GreB (1.0 μM) in 1× standard transcription buffer containing 0.2× RNAP diluent. Samples were incubated at 30°C, and at various times 5-μl aliquots were withdrawn and the reaction was stopped by the addition of two vol of 10 M urea loading buffer. The samples were run on 7 M urea/15% polyacrylamide gels and the amount of cleavage was determined on either an Ambis Radioanalytic Imager or a Molecular Dynamics PhosphorImager.

RESULTS

RNA Binding to *E. coli* RNA Polymerase. The CPG79 RNA interacts with *E. coli* RNA polymerase holoenzyme with a 1:1 stoichiometry and a K_d of ≈30 nM under the conditions we have used and results in the release of the σ subunit (data not shown) (19, 48). Fig. 1 shows the results of a mobility shift assay in which a constant amount of CPG79 RNA is incubated with increasing amounts of RNA polymerase. Even at enzyme excess, there is only a single shifted band, demonstrating the formation of a single complex. Scatchard analysis of the binding interaction also indicates a 1:1 stoichiometry. At a fixed concentration of RNA polymerase and RNA, an equivalent amount of complex is formed by either association or dissociation, indicating that an equilibrium process is involved (data not shown). Since both the association rate and dissociation rate in solution are extremely rapid ($t_{1/2} < 1$ min), the equilibrium between the complex and the free species is quickly established (C.R.A. and M.J.C., unpublished results).

Endonucleolytic Cleavage of RNA Requires Polymerase and Either GreA or GreB. Incubation of RNA polymerase prepared from *greA*$^-$ *greB*$^-$ cells, together with 5′-end-labeled CPG79 RNA and either GreA or GreB factor, leads to the formation of characteristic 5′-terminal cleavage products (Fig. 2). Formation of these products requires both the Gre factor and RNA polymerase (Fig. 2, lanes 14–16) and Mg^{2+} (see below) and is quantitatively inhibited by rifampicin. With GreA, cleavage of CPG79 RNA is nearly complete after 4 hr, and it gives primarily two 5′-end-labeled products of lengths about 55 and 57 nt, as judged by the piperidine ladder (there is a preferential cleavage next to G residues in this ladder, allowing facile indexing of products). With GreB, cleavage of CPG79 RNA is nearly complete after only 80 min, and only a single product of 70 nt is found. Hence, these reactions cannot be due to the contamination of either enzyme or factor preparations by an adventitious nuclease. The inhibition by rifampicin, in particular, suggests that cleavage is occurring in binary complexes, at or near the catalytic site of the enzyme.

Both the RNA polymerase preparation used and the samples of GreA and GreB contain traces of an adventitious nuclease. This nuclease gives small amounts of a characteristic 5′-terminal cleavage product of about 38 nt in the presence of GreA plus RNA polymerase (Fig. 2; see particularly lanes 4–7) and a 76-nt and two smaller products after 8 hr of incubation with either GreA of GreB alone (Fig. 2, lanes 13 and 14). Formation of these products is not inhibited by rifampicin and does not require both polymerase and the Gre factor.

Cleavage Depends on the RNA and Generates a 3′-OH. The sites, rate, and extent of cleavage depend on the RNA substrate, and a single RNA can be cleaved more than one time. When three different 5′-labeled RNA substrates were incubated under identical cleavage conditions for 8 hr, a

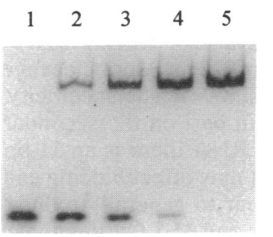

FIG. 1. Mobility shift of CPG79 RNA by *E. coli* RNA polymerase. CPG79 RNA (0.1 μM) was incubated with increasing amounts of RNA polymerase, and bound and free RNA were separated by 4% nondenaturing PAGE. The molar ratio of RNA polymerase to RNA was 0, 0.3, 0.75, 1.5, and 4.5 for lanes 1–5, respectively.

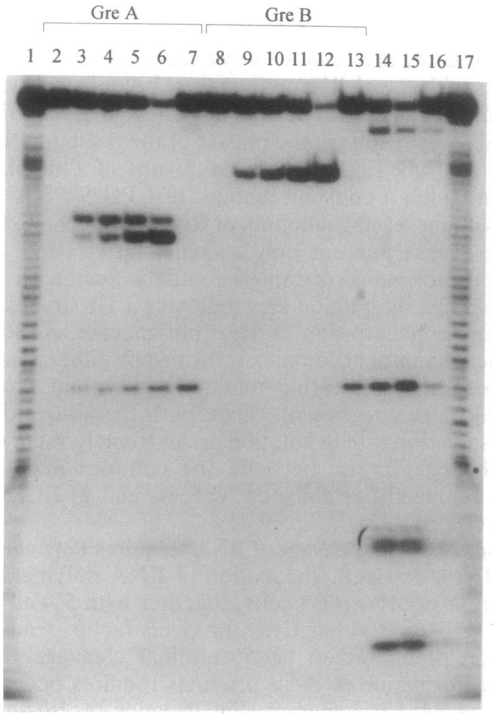

FIG. 2. Factor-dependent cleavage of CPG79 RNA by RNA polymerase. CPG79 RNA (40 nM) was incubated together with RNA polymerase (0.2 μM) and added factor (0.2 μM), and aliquots were removed at various times. Lanes 2–6, GreA time course at 0, 0.5, 1, 2, and 4 hr; lane 7, 4 hr in a mixture containing rifampicin at 0.02 mg/ml. Lanes 8–12, GreB time course at 0, 5, 10, 20, and 80 min; lane 13, 4 hr in a mixture containing rifampicin at 0.02 mg/ml. Lane 14, GreA (0.2 μM) alone without added polymerase at 8 hr. Lane 15, GreB (0.2 μM) without added polymerase at 8 hr. Lane 16, RNA polymerase (0.2 μM) without added factor, 20 hr. Lanes 1 and 17, piperidine cleavage ladders.

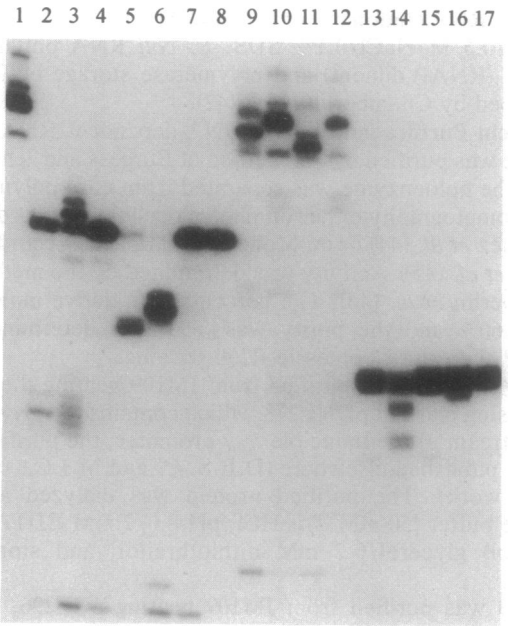

FIG. 3. Comparison of cleavage of three RNA substrates induced by GreA and GreB in the presence of RNA polymerase. CPG79 RNA (lanes 2–8), M19-68 RNA (lanes 1, 9–12), and N25-50 RNA (lanes 13–17) were incubated with RNA polymerase and either GreA (lanes 2–4, 9, 10, 14, 15) or GreB (lanes 5–7, 11, 12, 16, 17) for 8 hr. The RNA at time 0 is shown in lanes 1, 8, and 13, and lanes 4 and 7 show the effect of rifampicin at 0.02 mg/ml. After cleavage was allowed to proceed, ATP was added to 2.6 mM and the reaction mixture was further incubated for 2 hr (lanes 3, 6, 10, 12, 15, 17). Samples were stopped by the addition of 2 vol of 10 M urea loading buffer and run on 7 M urea/15% polyacrylamide gels. The sequence of M19-68 is AUCGAGAGGG AAGAGAAGAA GAGAGAGGCA CAGGCGAAUA GCCAUCCCAA UCGACACCGG GGUCCGGG. The sequence of N25-50 RNA is AUAAAUUUGA GAGAGGAGUU UAAAUAUGGC UGGUUCUCGU AGAAAGAAAC.

unique pattern of 5′-terminal products was generated for each RNA that depended on both the identity of the substrate and the added factor (Fig. 3). The GreA-induced cleavage patterns for CPG79, M19-68, and N25-50 RNA after 8 hr are shown in lanes 2, 9, and 14. Comparison of each substrate shows that the extent and sites of cleavage vary considerably among the three substrates. Cleavage induced by GreB after 8 hr generates a different set of products, which also depends on the substrate (lanes 5, 11, 15). In addition, the rates can vary considerably on the same substrate RNA, depending on which factor is included (compare lanes 2 with 5, 9 with 11, and 14 with 16).

The study of cleavage of 5′-end-labeled RNAs can measure the extent of shortening of a cleaved RNA. By using 3′-end-labeled RNA, however, we can determine the size of the 3′ product of the initial cleavage event. In general, when 3′-end-labeled RNA is used, cleavage by either GreA or GreB results in the release of a 1- to 3-nt labeled product. However, GreA-induced cleavage of CPG79 RNA releases a ≈23-nt fragment, while GreB-dependent cleavage results in an 8-nt product (data not shown). The specificity of RNA cleavage probably depends in part on the secondary structure of the RNA; for pCPG79 RNA there is an 11-bp hairpin 9 nt from the 3′ terminus that may affect binding and cleavage (Fig. 4). RNA hairpins appear to generally reduce binding in binary complexes (C.R.A. and M.J.C., unpublished data), and further studies are needed to determine the actual structure of the RNA in those complexes.

The finding that binary complexes can carry out the transcript cleavage reaction suggested that the RNA in such complexes is bound to the enzyme along the 3′ end and that the catalytic site of the enzyme is positioned at or near the 3′-OH end of the RNA. This led us to ask whether these complexes could add nucleotides to the 3′-OH end of the bound RNA, as is seen for ternary complexes. We found that binary complexes of RNA polymerase and RNA will catalyze limited nucleotide addition to the RNA 3′-OH end in a template-independent, rifampicin-sensitive manner. We have

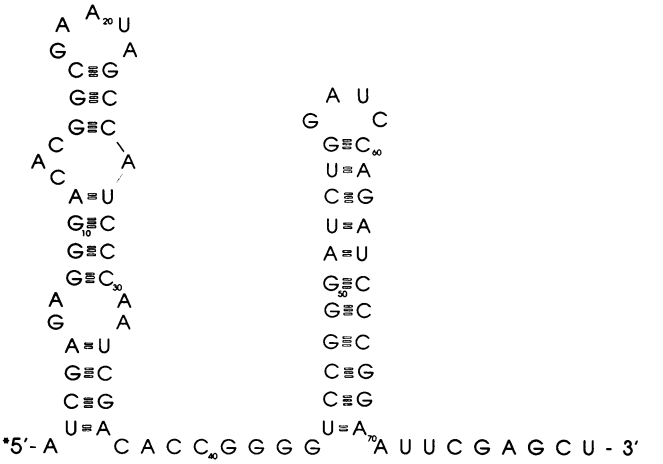

FIG. 4. Predicted secondary structure of CPG79 RNA. CPG79 RNA was folded by the method of Zuker (50). This predicted structure is consistent with RNase mapping studies (C.R.A., unpublished studies).

used this observation to demonstrate that the endonucleolytic cleavage of RNA induced by both GreA and GreB results in the formation of a 3'-OH end (Fig. 3). After GreA or GreB induced cleavage, ATP was added and the reaction mixture was incubated for an additional 2 hr. The rate and extent of template-independent nucleotide addition depend on the RNA substrate.

Nucleotide addition after GreA-induced cleavage is shown for CPG79 RNA, M19-68 RNA, and N25-50 RNA (Fig. 3, lanes 3, 10, and 15 respectively). Nucleotide addition to GreB-cleaved RNA is shown in Fig. 3, lanes 6, 12, and 17. For CPG79 RNA, nucleotide addition occurs for both the cleaved products and the initial substrate (compare lanes 2 and 3 in Fig. 3). In contrast, under these conditions, intact N25-50 RNA is not elongated and the elongation of the cleaved products is limited (Fig. 3, lanes 15 and 17). Limited elongation is also observed for intact M19-68 RNA as well as the GreB-induced cleavage product of CPG79 RNA (Fig. 3, lanes 4, 10, 12). Nucleotides can also have an effect on the cleavage reaction either indirectly by generating a more susceptible RNA substrate through nucleotide addition that cleaves more rapidly or through a direct effect of the nucleotide on cleavage (compare lanes 5 and 6 in Fig. 3). It is possible that this binary addition reaction explains the phenomenon in which nontemplated RNA nucleotides are found at the 3' termini that has been seen with *E. coli*, T7, and SP6 RNA polymerases (49, 51, 52).

Cleavage Requires Mg^{2+} and Association with RNA Polymerase. The cleavage reaction depends on the presence of a divalent cation and is inhibited by agents that interfere with the interaction of the RNA with RNA polymerase. When Mg^{2+} is excluded from the reaction, no cleavage is observed. The addition of Mg^{2+} up to 1.0 mM results in cleavage, while amounts above this progressively inhibit the reaction by directly interfering with the interaction of the RNA with the enzyme (C.R.A. and M.J.C., unpublished results). The binding of CPG79 RNA to the enzyme does not require Mg^{2+} however, suggesting an essential role of Mg^{2+} in the catalytic event. Alternatively, Mg^{2+} may be essential for proper interactions near the active site, while other interactions play the dominant role for the observed binding in its absence. Spermidine, salt, and heparin all inhibit the binary cleavage reaction. Since each of these also inhibits the binding of RNA to RNA polymerase as determined by filter binding assays, we presume that this is the mode of action in this case as well (refs. 15 and 53; C.R.A. and M.J.C., unpublished work). Neither GreA nor GreB shows any detectable nucleic acid binding activity by either filter binding or mobility shift assay (data not shown) (25, 26). Two antagonists of transcription elongation—streptolydigin, which blocks phosphodiester bond formation (54, 55), and ppGpp, which increases pausing (56)—were unable to affect the reaction even at high concentration (data not shown).

DISCUSSION

Early studies of RNA binding to *E. coli* RNA polymerase showed that binding had a stoichiometry of 1:1 and led to the release of the σ factor (14, 19, 23). Our results are consistent with these published studies. However, we note some kinetic and thermodynamic differences. In our studies, we have found RNA binding to *E. coli* RNA polymerase to be in a rapid equilibrium, which contrasts with some previous observations (14, 19, 23). These differences are likely to be due to the extensive secondary and tertiary structure of tRNA used in the previous work, since both sets of experiments were performed under similar conditions. In studies to be published elsewhere, we have shown that maximum binding of homopolymers occurs only after 25–35 nt, suggesting a large binding site, or two smaller sites separated from each other located at or near the 3' end of the RNA. Binding to end-labeled RNA populations is also consistent with binding near the 3'-OH end, and it also suggests a large binding site (C.R.A. and M.J.C., unpublished results). The presumption of binding near the 3'-OH end of the RNA is confirmed by the finding that bound RNA can undergo Gre factor-dependent cleavage at the 3' end and can serve as an acceptor for nucleotides from NTP. Both of these reactions had previously been thought to occur only in ternary DNA·RNA·enzyme complexes. Similar results have been obtained with *Saccharomyces cerevisiae* RNA polymerase II and the transcript cleavage factor TFIIS (57). These findings provide strong evidence that the RNA in binary complexes is bound to the enzyme in a form related to the mode of binding in ternary transcription complexes.

There are several differences between the cleavage of RNA in binary and ternary complexes. The rate of cleavage is generally much slower in binary complexes than in ternary complexes (refs. 24–26; D.E.S.-C., unpublished studies). This slower rate may result from the fact that the RNA in the binary complex is bound in an equilibrium, while that in the ternary complexes is normally not dissociable under physiological conditions. In addition, while we have not observed cleavage in the absence of added factor for the binary complex, polymerase purified from a strain deleted for both of the known Gre factors is still able to induce cleavage in some ternary complexes, though with slightly different kinetics and specificity (D.E.S.-C., unpublished observations). Thus, while RNA binding in a binary complex has some properties in common with binding in a ternary complex, it is also likely that the actual structure of ternary complexes is altered through DNA binding as well.

Similarities between the cleavage of RNA in binary complexes and that in ternary complexes include the requirement for Mg^{2+} and the generation of products with 3'-OH and 5'-PO_4 termini. The size of the cleavage products produced with GreA and GreB depends both on the factor and on the RNA involved. Cleavage of ternary complexes with GreB can give 3'-end products of 1–10 nt, while cleavage with GreA normally gives products of 1–3 nt. Cleavage of binary complexes with GreB gives 3'-end products of up to 9 nt, consistent with its mode of cleavage in ternary complexes. In general, GreA-dependent cleavage in binary complexes gives 1- to 3-nt products; however, cleavage of the CPG79 RNA gives an ≈23-nt product, quite different from any cleavage reported for ternary complexes. Studies directly comparing cleavage of identical RNAs in both binary and ternary complexes may provide important information on the specificity of the cleavage reaction. These results suggest that the RNA·enzyme complex is the substrate for cleavage of both binary and ternary complexes.

It is interesting to find that rifampicin can block the initial cleavage event in the binary complex. Both Mi and Hartman (58) and Shulz and Zillig (59) have shown that binding of rifampicin is competitive with binding short oligomers to the RNA polymerase, suggesting that rifampicin might block binding near the 3'-OH end by binding in the RNA site. However rifampicin has no strong destabilizing effect on binding of longer RNA (C.R.A. and M.J.C., unpublished results). The inhibition of cleavage by rifampicin could be due to a steric hindrance of RNA binding at an essential point in the binding site. In particular, rifampicin may block essential interactions near the active site. Alternatively, rifampicin binding may bring about an indirect alteration of the conformation of the RNA binding site either by preventing the binding of the 3' terminus or by preventing the movement of the catalytic site.

Recent studies by Rudd, M. Izban, and D. Luse (personal communication) provide evidence that the transcript cleavage reaction, at least with RNA polymerase II at natural

arrest sites, is catalyzed by the catalytic site of the RNA polymerase itself. They find that TFIIS-induced hydrolytic cleavage within an arrested ternary complex is replaced by pyrophosphorolytic cleavage when pyrophosphate is provided in the reaction. If this is true for the *E. coli* RNA polymerase, it provides direct evidence that the catalytic site of the enzyme can move independently of the 3′-OH terminus of the RNA transcript. Since GreA can cleave at quite different sites than GreB, this would suggest that the cleavage factors can alter the position of the catalytic site along the RNA chain.

We have recently proposed a model for how RNA polymerase is able to elongate RNA chains that differs from the "minimalist" model of Gamper and Hearst (1) and Yager and von Hippel (3). In this model, the RNA polymerase is bound to DNA through two different DNA binding sites, which can alternately lock and slide, giving rise to an inchworm-like movement along DNA. The model invokes two similar RNA binding sites of 8–10 nt each as the primary mode of RNA binding to the RNA polymerase. This model presumes that there is normally only a short DNA·RNA hybrid formed (2–3 nt) and that this hybrid does not contribute significantly to the stability of RNA binding. Our current results fit the general predictions of this model quite closely. In particular, DNA plays no obligatory role in the correct binding of RNA to the polymerase. Similarly, the model predicts that cleavage and nontemplated nucleotide addition might well occur in such complexes. Finally, a major prediction of the inchworm model is that the catalytic site of the polymerase could move independently of the 3′-OH terminus of the RNA, and this now appears to be confirmed as well.

In independent studies, Liu *et al.* (60) have shown that the T4 replication machinery is able to pass *E. coli* RNA polymerase on the same template strand without causing its release. This would seem to provide excellent independent confirmation that RNA in such ternary complexes is bound primarily to the protein and that any DNA–RNA interactions can be transiently broken, without loss of RNA from the ternary complex.

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